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Enhanced glucose uptake via GLUT4 fuels recovery from calcium overload after ischaemia–reperfusion injury in sevoflurane- but not propofol-treated hearts

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Editor's key points

- Changes in energy metabolism in response to sevoflurane, propofol, or Intralipid were measured in working rat hearts after ischaemia and reperfusion.
- Sevoflurane, but not propofol, improved the recovery of heart function and decreased calcium overload via enhanced glucose uptake.
- This study begins to explain the mechanisms behind the cardioprotective effects of sevoflurane.

Background. So far, no study has explored the effects of sevoflurane, propofol, and Intralipid on metabolic flux rates of fatty acid oxidation (FOX) and glucose oxidation (GOX) in hearts exposed to ischaemia–reperfusion.

Methods. Isolated paced working rat hearts were exposed to 20 min of ischaemia and 30 min of reperfusion. Peri-ischaemic sevoflurane (2 vol%) and propofol (100 μM) in the formulation of 1% Diprivan® were assessed for their effects on oxidative energy metabolism and intracellular diastolic and systolic Ca^{2+} concentrations. Substrate flux was measured using [^{3}H]palmitate and [^{14}C]glucose and [Ca^{2+}] using indo-1AM. Western blotting was used to determine the expression of the sarcolemmal glucose transporter GLUT4 in lipid rafts. Biochemical analyses of nucleotides, ceramides, and 32 acylcarnitines were also performed.

Results. Sevoflurane, but not propofol, improved the recovery of left ventricular work (P=0.008) and myocardial efficiency (P=0.008) compared with untreated ischaemic hearts. This functional improvement was accompanied by reduced increases in post-ischaemic diastolic and systolic intracellular Ca^{2+} concentrations (P=0.008). Sevoflurane, but not propofol, increased GOX (P=0.009) and decreased FOX (P=0.019) in hearts exposed to ischaemia–reperfusion. GLUT4 expression was markedly increased in lipid rafts of sevoflurane-treated hearts (P=0.016). Increased GOX closely correlated with reduced Ca^{2+} overload. Intralipid alone decreased energy charge and increased long-chain and hydroxyacylcarnitine tissue levels, whereas sevoflurane decreased toxic ceramide formation.

Conclusions. Enhanced glucose uptake via GLUT4 fuels recovery from Ca^{2+} overload after ischaemia–reperfusion in sevoflurane- but not propofol-treated hearts. The use of a high propofol concentration (100 μM) did not result in similar protection.

Keywords: anaesthetics inhalation, sevoflurane; anaesthetics i.v., propofol; energy metabolism; myocardial reperfusion injury; rats

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Cardiac metabolism critically affects contractile function in acute and chronic disease states.[1] Pronounced metabolic shifts in substrate preference, namely between glucose oxidation (GOX) and fatty acid oxidation (FOX), occur in obesity and diabetes, heart failure, and ischaemia–reperfusion injury. From multiple previous metabolic studies of the heart, the prevailing concept suggested that favouring economic (i.e. ATP-sparing) glucose over FOX is especially beneficial in stressed hearts with limited oxygen supply.[2] Using the aerobically perfused working rat heart model, we recently showed in an extensive metabolic analysis that sevoflurane, propofol, and its solvent Intralipid elicit distinct metabolic profiles in the myocardium,[3] which may have important implications for the already jeopardized diseased heart. In that and previous studies, we demonstrated that sevoflurane reduces FOX by redistributing the principal fatty acid transporter CD36 from lipid rafts to non-functional intracellular pools (short-term effects)[3] and by decreasing peroxisome

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proliferator-activated receptor-regulated gene products involved in FOX (long-term effects).[4] In contrast, propofol (100 μM) increases GOX by dephosphorylating the pyruvate dehydrogenase (PDH) complex, elevates formation of lipotoxic ceramides, and inhibits 3-hydroxy-acyl-coenzyme A (CoA) dehydrogenase, a critical enzyme of mitochondrial β-oxidation.[3] So far, however, no study has directly measured the metabolic effects of sevoflurane and propofol on flux rates of glucose and fatty acids in hearts subjected to ischaemia–reperfusion injury. On the basis of our previous results, we hypothesized that the two anaesthetics for which cardioprotective properties were reported in the past[5–8] would differentially modulate cardiac metabolism under ischaemia–reperfusion conditions and that changes in metabolism could be related to measured intracellular Ca^{2+} overload. Beat-to-beat Ca^{2+} recordings and contractility in the working rat heart model served as outcome measures and were related to changes in energy metabolism and metabolites.

Methods

Working heart perfusion protocols and beat-to-beat intracellular Ca^{2+} measurements

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the experimental protocol used in this investigation was approved by the University of Alberta Animal Policy and Welfare Committee. Male Sprague–Dawley rats (350–400 g), treated according to the guidelines of the University of Alberta Animal Policy and Welfare Committee and the Canadian Council on Animal Care, were killed with an overdose of pentobarbital (150 mg kg^{-1}, i.p.). Each heart was rapidly removed and perfused initially (15 min) in a non-working Langendorff mode with the Krebs–Henseleit solution. Working mode perfusion was subsequently established (11.5 mm Hg preload, 80 mm Hg afterload, 5 Hz) with a recirculating perfusate (100 ml, 37°C, pH 7.4, gassed with 95% O_2/5% CO_2) that consisted of a modified Krebs–Henseleit solution containing (mM): KCl (4.7), NaCl (118), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $CaCl_2$ (2.5), $NaHCO_3$ (25), glucose (11), palmitate (1.2, pre-bound to 3% bovine serum albumin), and insulin 100 mU $litre^{-1}$. To determine beat-to-beat intracellular Ca^{2+} concentrations, hearts were loaded with the fluorescent $[Ca^{2+}]$ indicator indo-1AM (5 μM, 25 min) in the working mode perfusion. After a washout period of 5 min in the Langendorff mode, the working mode was re-established with a fresh modified Krebs–Henseleit solution using a second perfusion circuit. With the aid of a random table, hearts were assigned to the four treatment groups (Supplementary Fig. S1) (n=6 for each group): (i) time-matched perfusion without treatment (IR), (ii) sevoflurane[8] (SEVO+IR) administered at 2 vol%, (iii) propofol 100 μM (PROP100+IR) in the formulation of Diprivan® 1% (AstraZeneca Inc., Mississauga, Ontario, Canada), or (iv) the corresponding Intralipid control (INTRA100+IR). A concentration of 100 μM was chosen for propofol since previous studies showed a clear dose–response for its cytoprotection, with 100 μM having the most pronounced protection in hearts.[6] All hearts were subjected to 20 min of 37°C zero-flow ischaemia and 30 min of reperfusion and then immediately frozen in liquid nitrogen and stored at −80°C for subsequent analyses. Indo-1 fluorescence was measured from a small area (~0.3 cm^2) of the epicardial surface of the left ventricle using a spectrofluorometer (Photon Technology International, London, Ontario, Canada). Signals were acquired at 500 Hz, and the ratio of indo-1 fluorescence emitted at 405 and 485 nm (F_{405}/F_{485} ratio) was calculated to provide an index of intracellular $[Ca^{2+}]$.[9] Cardiac output (ml min^{-1}) and aortic flow (ml min^{-1}) were measured using ultrasonic flow probes (Transonic T206, Transonic Systems Inc., Ithaca, NY, USA). Left ventricular work (LVW; ml min^{-1} mm Hg), coronary flow (ml min^{-1}), and coronary vascular conductance (ml min^{-1} mm Hg^{-1}) were calculated as described previously.[9] Measurements of mechanical functions were averaged for the pre- and post-ischaemic period. Myocardial efficiency was defined as the ratio of LVW per produced acetyl-CoA per gram dry weight (dry wt).

Metabolic flux measurements: determination of GOX and FOX

Rates (expressed as μmol g^{-1} dry wt min^{-1}) of GOX and FOX were measured by determining $^{14}CO_2$ production and ^3H_2O production in hearts perfused with [U-^{14}C]glucose and [9,10-^{3}H]palmitate, respectively.[10] Samples were obtained every 10 min and rates were calculated for each time interval and were averaged for the pre- and post-ischaemic period. Subsequently, the difference between averaged preischaemic and averaged post-ischaemic values was computed.

Determination of nucleotides and ceramides

Adenine nucleotides and ceramides were measured after tissue extraction using high-performance liquid chromatography, as described previously.[3] The energy charge of the adenylate pool was computed as (ATP+0.5 ADP)/(ATP+ADP+AMP).

Mass spectrometry for acylcarnitine profiling

From four randomly chosen hearts of each group, tissue levels of 32 acylcarnitine species were measured using electrospray ionization tandem mass spectrometry.[3] Acylcarnitines were extracted from heart tissue with methanol and quantified using eight isotopically labelled internal standards (Cambridge Isotopes Laboratories, Andover, MA, USA). Precursor ions of m/z 85 in the mass range of m/z 150–450 were acquired on a PE SCIEX API 365 LC-ESI-MS/MS instrument (Applied Biosystems, Foster City, CA, USA).

Determination of tissue triglyceride content and [9,10-^{3}H]palmitate incorporation

After chloroform/methanol extraction of lipids from cardiac tissue, triglyceride content was quantified colorimetrically

with the enzymatic assay kit L-Type Triglyceride M (Wako Pure Chemical Industries, Richmond, VA, USA). Incorporation of [9,10-^{3}H]palmitate into triglycerides was counted.[3]

Determination of tissue glycogen content and [U-^{14}C]glucose incorporation

Glycogen content (μmol glucosyl units g^{-1} dry wt) was determined by powdering heart tissue samples and subjecting them to alkaline extraction with 30% KOH followed by ethanol precipitation and acid hydrolysis (2 N H_2SO_4) and analysis of glucose content. Incorporation of [U-^{14}C]glucose into glycogen was counted.[3]

PDH complex activity assay

PDH activity was measured by the radioisotopic-coupled enzyme assay which determines the ratio of PDH_{active}/PDH_{total}.[11]

Immunoblotting for GLUT4 expression in lipid rafts and phosphorylated AMP-activated protein kinase/AMP-activated protein kinase ratio

Whole tissue extracts and detergent-free fractions 2 and 3 of lipid rafts/caveolae containing GLUT4 were prepared from the five IR and five SEVO+IR hearts from which complete Ca^{2+} and metabolic data were obtained, and western blotting was performed as described previously.[3] The phosphorylated AMP-activated protein kinase (AMPK)/AMPK ratio was determined in four randomly chosen hearts. The following primary antibodies were used: rabbit anti-GLUT4 (Cell Signaling Technology, Danvers, MA, USA), mouse anti-flotillin (610820, BD Biosciences, Mississauga, Ontario, Canada), rabbit anti-AMPK (2532, Cell Signaling Technology), rabbit anti-pY172-AMPK (2531, Cell Signaling Technology), and rabbit anti-α-actin (I-19, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Protein band intensities were quantified by ImageJ software [http://rsbweb.nih.gov/ij/ (accessed December 1, 2008)].

Statistical analysis

The sample size was calculated based on previously reported data for the recovery of left ventricular function and changes in GOX and diastolic Ca^{2+} concentrations.[9] With an expected difference of 50% between group means, 20% SD of the means, the significance level $\alpha=0.05/3$ (Bonferroni's correction for three groups since PROP100+IR was compared with IR and INTRA100+IR), and $\beta=0.8$, a sample size of five hearts per group was necessary. The significance of differences in haemodynamic and metabolic variables among groups was determined by Student's *t*-test (two groups) or by analysis-of-variance (ANOVA) followed by the Student–Newman–Keuls method for *post hoc* analysis (three groups) or by non-parametric methods (Wilcoxon–Mann–Whitney rank-sum test and Kruskal–Wallis analysis-of-variance on ranks) depending on the underlying data distribution. To test the association between GOX and changes in intracellular Ca^{2+} concentrations, linear regression analysis was performed. The correlation coefficient *R*, the standard error of the estimate, and the corresponding *P*-value are reported. Differences are considered significant if $P<0.05$. SigmaStat (version 3.5; Systat Software, Inc., Chicago, IL, USA) was used for the analyses.

Results

Sevoflurane but not propofol reduces Ca^{2+} overload and increases myocardial efficiency after ischaemia–reperfusion injury in working hearts

During baseline aerobic perfusion, LVW was stable and similar among groups. Upon initiation of global no-flow ischaemia, all detectable left ventricular mechanical function ceased virtually immediately (<60 s). After 20 min of zero-flow

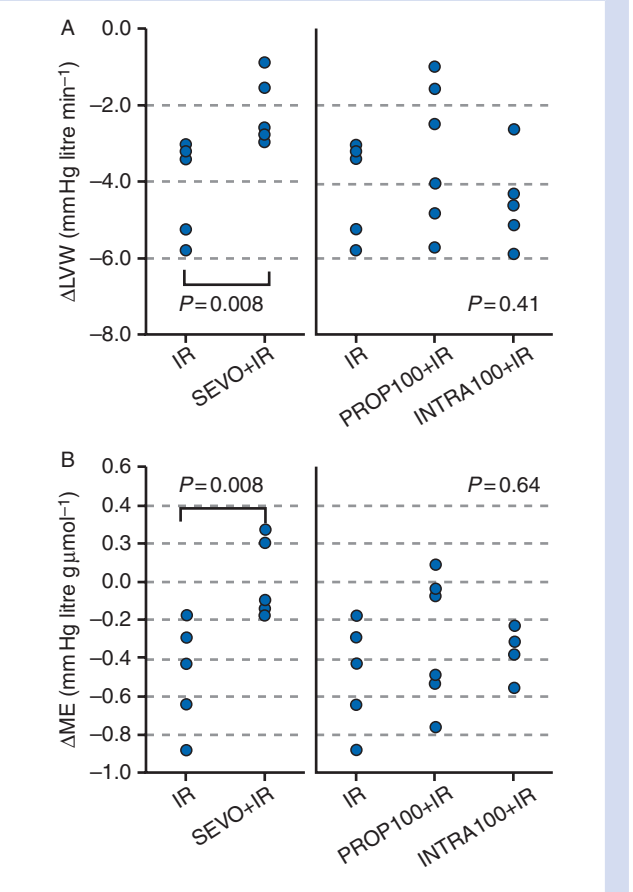

Fig 1 Changes in LVW (ΔLVW, A) and myocardial efficiency (ΔME, B). IR, hearts exposed to ischaemia–reperfusion; SEVO+IR, hearts exposed to ischaemia–reperfusion and treated with 2 vol% sevoflurane before and after ischaemia; PROP100+IR, hearts exposed to ischaemia–reperfusion and treated with 100 μM propofol before and after ischaemia; INTRA100+IR, the corresponding Intralipid control group. Complete haemodynamic data were obtained for six hearts in each group except for IR and INTRA100+IR with only five hearts (also see Supplementary Table S1). Complete metabolic data were obtained in six hearts of each group except for IR and SEVO+IR with five hearts and INTRA+IR with four hearts. *P*-values were computed using non-parametric tests.

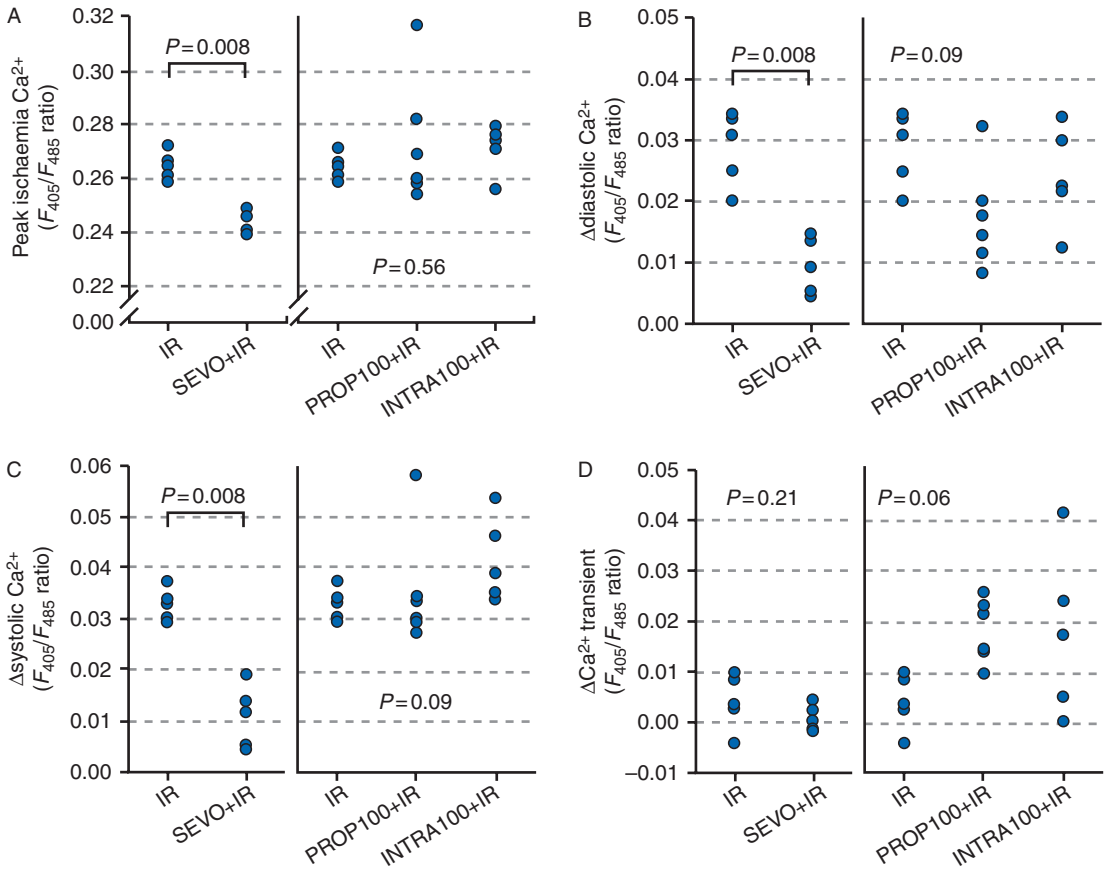

Fig 2 Intracellular Ca^{2+} concentrations at the end of ischaemia (A) and at the end of reperfusion (diastolic, B; systolic, C; and transient amplitude, D) in untreated hearts (IR, $n=5$) or in hearts that were exposed to sevoflurane (2 vol%, SEVO+IR, $n=6$), propofol (100 μM, PROP100+IR, $n=6$), or Intralipid (INTRA100+IR, $n=5$). *P*-values were computed using non-parametric tests.

ischaemia and 30 min of reperfusion, the decrease in LVW ($P=0.008$), peak systolic pressure ($P=0.035$), and stroke volume ($P=0.016$) was significantly smaller in sevoflurane-treated hearts when compared with untreated control hearts (Fig. 1A; Supplementary Table S1). In contrast, propofol was not protective when compared with untreated control hearts or Intralipid ($P=0.41$). Myocardial efficiency was higher in sevoflurane-treated hearts ($P=0.008$), but was depressed to a similar extent in propofol-treated and untreated hearts ($P=0.64$; Fig. 1B). During global no-flow ischaemia, Ca^{2+} transients ceased within 5 min and the average intracellular Ca^{2+} concentrations (termed diastolic Ca^{2+}), an index of ATP depletion and ischaemic contracture and injury, were lowest in sevoflurane-treated hearts ($P=0.008$) but were unchanged in propofol- or Intralipid-treated hearts ($P=0.56$; Fig. 2A). Diastolic ($P=0.008$; Fig. 2B) and systolic Ca^{2+} concentrations ($P=0.008$; Fig. 2C) measured during reperfusion were decreased in sevoflurane-treated hearts when compared with untreated control hearts. In contrast, propofol did not significantly decrease Ca^{2+} overload during reperfusion (Δdiastolic Ca^{2+}, $P=0.09$; Δsystolic Ca^{2+}, $P=0.09$; Fig. 2; Supplementary Table S2).

Sevoflurane but not propofol increases GOX after ischaemia–reperfusion injury in working hearts

Determinations of GOX, FOX, and Krebs cycle acetyl-CoA production rates after ischaemia–reperfusion injury revealed an increase in GOX in sevoflurane-treated hearts when compared with untreated control hearts ($P=0.009$) (Fig. 3A; Supplementary Table S3). In accordance with our previous report, sevoflurane-treated hearts also showed a significant decrease in FOX ($P=0.016$; Fig. 3B) and a corresponding decline in acetyl-CoA production ($P=0.03$; Fig. 3C). Lower peak ischaemic Ca^{2+} concentrations ($P=0.002$) and changes in diastolic ($P=0.002$) and systolic ($P=0.005$) Ca^{2+} concentrations were closely correlated with higher GOX (Fig. 4). No changes in GOX ($P=0.45$), FOX rate ($P=0.43$), or acetyl-CoA turnover ($P=0.45$) were observed in propofol- and Intralipid-treated hearts during reperfusion (Fig. 3; Supplementary Table S3). Levels of triglycerides were markedly reduced in sevoflurane- ($P=0.044$) but not propofol-treated hearts ($P=0.51$; Supplementary Table S4), suggesting reduced fatty acid uptake, as previously shown.[3]

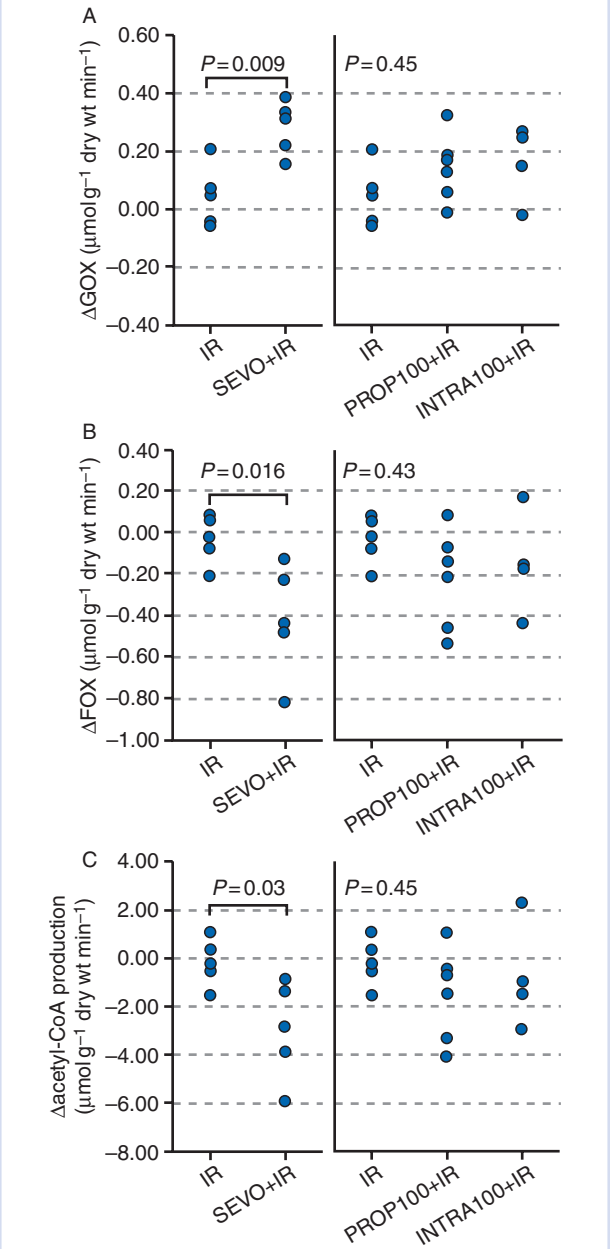

Fig 3 Changes in rates of GOX (ΔGOX, A), FOX (ΔFOX, B), and acetyl-CoA production (C) during reperfusion in untreated hearts (IR, $n=5$) or in hearts that were exposed to sevoflurane (2 vol%, SEVO+IR, $n=5$), propofol (100 μM, PROP100+IR, $n=6$), or Intralipid (INTRA100+IR, $n=4$). *P*-values were computed using non-parametric tests.

Sevoflurane increases GLUT4 expression in lipid rafts in an AMPK-independent manner after ischaemia–reperfusion injury in working hearts

To further explore the mechanism of the sevoflurane-induced increase in GOX, the expression of functional, that is, lipid raft-associated GLUT4 was determined in working hearts exposed to ischaemia–reperfusion. Sevoflurane-treated hearts exhibited higher GLUT4 expression in lipid rafts when compared with untreated control hearts ($P=0.019$; Fig. 5A), which was not related to increased activity of AMPK ($P=0.72$; Fig. 5B). Also, sevoflurane did not change the activity of the PDH complex (PDC; $P=0.22$; Fig. 5C). Total glycogen content and radioactively labelled glycogen were not different among groups (Supplementary Table S4). Periischaemic administration of sevoflurane ($P=0.009$) but not propofol ($P=0.28$) markedly reduced ceramide formation (Supplementary Fig. S2). Intralipid administration increased long-chain acylcarnitines ($P\leq0.023$) and hydroxyacylcarnitines ($P=0.027$; Supplementary Tables S5 and S6) and markedly reduced the cardiac energy charge ($P=0.012$; Supplementary Table S7).

Discussion

In this study, we used the working rat heart model, which allows the measurement of metabolic flux rates under carefully controlled conditions of energy substrate supply and physiological workload. Under aerobic conditions, more than 70% of cardiac energy requirements is provided by fatty acids[1 12] with the remainder coming from glucose and to a much lesser degree from lactate/pyruvate or amino acids. The inclusion of palmitate in the perfusate is an important aspect of this study as it permits assessment of myocardial substrate preference, a key determinant of the ability of cardiac muscle to recover after ischaemia. During ischaemia–reperfusion, metabolic flexibility (i.e. switching from one to the alternative substrate) is virtually lost whereby the more rapid recovery of FOX inhibits the rates of GOX.[13 14] The results of our study now provide evidence that sevoflurane, in contrast to propofol, reduces FOX and concomitantly increases GOX during reperfusion. These notions extend our recent findings from aerobically perfused working hearts, where we demonstrated that sevoflurane reduces FOX by decreasing fatty acid uptake at the sarcolemma and increases net GOX.[3] There is ample evidence of a close and causal relationship between cardiac metabolism and contractility, mainly from clinical studies in patients with inherited deficiencies of metabolic enzymes[15] and more recently from studies with genetically modified mice.[16] Moreover, effects of metabolic interventions on mechanical function support the concept that energy metabolism indeed is a critical factor in the haemodynamic deterioration of the failing heart. Overexpression of GLUT1, the glucose transporter responsible for basal insulin-independent cellular glucose uptake, delays progression to failure after aortic constriction in murine hearts.[16] On the other hand, inhibition of FOX via sarcolemmal (CD36)[17] or mitochondrial (CPT1) fatty acid uptake[18] or at the level of β-oxidation[19] may improve mechanical work in the stressed heart. Stimulation of GOX directly or indirectly (i.e. by inhibition of FOX)[1] has reportedly salutary economic effects on cardiac metabolism. First, the oxygen cost of fatty acid as substrate is ∼11% higher compared with glucose since FOX shifts a greater proportion of electrons to

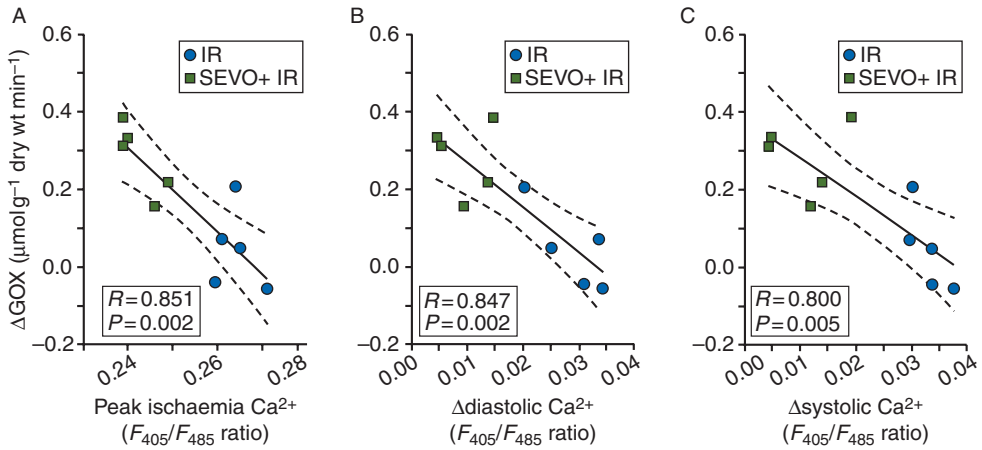

Fig 4 Regression plots with 95% confidence intervals for correlations between changes in GOX rates and peak Ca^{2+} concentrations during ischaemia (A: $R=0.851$; standard error of estimate=0.087), changes in diastolic (B: $R=0.847$; standard error of estimate=0.088), and systolic (C: $R=0.800$; standard error of estimate=0.099) Ca^{2+} concentrations for untreated hearts (IR) or for hearts exposed to sevoflurane (2 vol%, SEVO+IR).

ubiquinone complex II of the respiratory chain.[20] Also, four-carbon units as derived from glucose metabolism increase the concentrations of citric acid cycle intermediates allowing more efficient oxidation of two-carbon units. Secondly, FOX couples less efficiently electron with proton flux ('uncoupling') in mitochondria, thereby increasing the formation of reactive oxygen species.[21] Thirdly, increased FOX uncouples glycolysis from GOX and enhances H^+ formation.[22] We have previously reported on the physiological importance of sevoflurane-mediated fuel shifts in patients undergoing off-pump coronary artery bypass graft surgery.[4] In that study, sevoflurane-induced attenuation of transcripts involved in FOX closely correlated with improved cardiac contractility. The results of the present study extend our previous findings and reveal that the long-term transcriptional metabolic remodelling by sevoflurane is indeed a true mirror of its short-term metabolic phenotype.

In the current study, sevoflurane-mediated increase in GOX is likely to be the result of increased GLUT4 expression in lipid rafts, since the activity of the master switch enzyme of GOX, the PDC, was unaffected by sevoflurane. GLUT4 is the insulin-sensitive glucose transporter of the heart, which is translocated from intracellular compartments, mainly endosomes, to sarcolemmal caveolae,[23] in response to increased cardiac contractility, insulin stimulation, or activation of AMPK due to metabolic stress.[24 25] Our results show that the increased GLUT4 expression in sevoflurane-treated hearts is independent of AMPK activation, but since GLUT4 translocation is contractility-dependent, the increased GLUT4 expression in sevoflurane-treated hearts could be a consequence of the improved contractility rather than a cause. However, the ability of sevoflurane in aerobically perfused hearts to relatively increase net GOX by decreasing FOX, while maintaining GLUT4 expression in lipid rafts at a lower contractility (negative inotropy), rather implies that enhanced glucose uptake and oxidation may be at least partly contributing to the improved functional recovery in sevoflurane-treated hearts. Inhibition of fatty acid uptake and oxidation are known to increase GLUT4 expression via the glucose–fatty acid cycle ('Randle cycle').[1 26] Moreover, long-chain CoAs and triglycerides may activate several serine kinases that inhibit insulin action.[27] In our study, sevoflurane also decreased the formation of ceramides, which are known to mediate insulin resistance by inhibiting protein kinase B.[28] Inhibition of serine palmitoyltransferase, the rate-limiting enzyme in ceramide synthesis, reduces FOX but concomitantly increases GOX.[29] During ischaemia–reperfusion, ceramides also accumulate in lipid rafts,[30] which might affect GLUT4 function. Using microdialysis probes, Carles and colleagues[31] showed a better availability of glucose in the skeletal muscle of patients during ischaemia and reperfusion after sevoflurane exposure. This is in line with observations from skeletal muscle cells, showing that sevoflurane enhances glucose uptake through activation of tyrosine kinases.[32] Collectively, sevoflurane potentially improves insulin signalling in the heart. In our study, sevoflurane also markedly reduced the accumulation of triglycerides and ceramides in the post-ischaemic heart. Beside their insulin-antagonistic effects, ceramides promote inflammation, increase mitochondrial Ca^{2+}, and impair mitochondrial respiration.[33 34] On the other hand, long-chain and hydroxyacylcarnitines, typical markers of a dysfunctional fatty acid metabolism,[35] accumulated in Intralipid-treated hearts. Acylcarnitines are increased in ischaemic hearts due to inhibition of β-oxidation and may worsen intracellular Ca^{2+} overload.[36] Amphiphilic acylcarnitines are further capable of inhibiting mitochondrial ATP-dependent potassium channels[37] and 'acylate' essential metabolic enzymes. Hence, it is not surprising that we observed a significant decrease in energy charge in Intralipid-treated hearts.

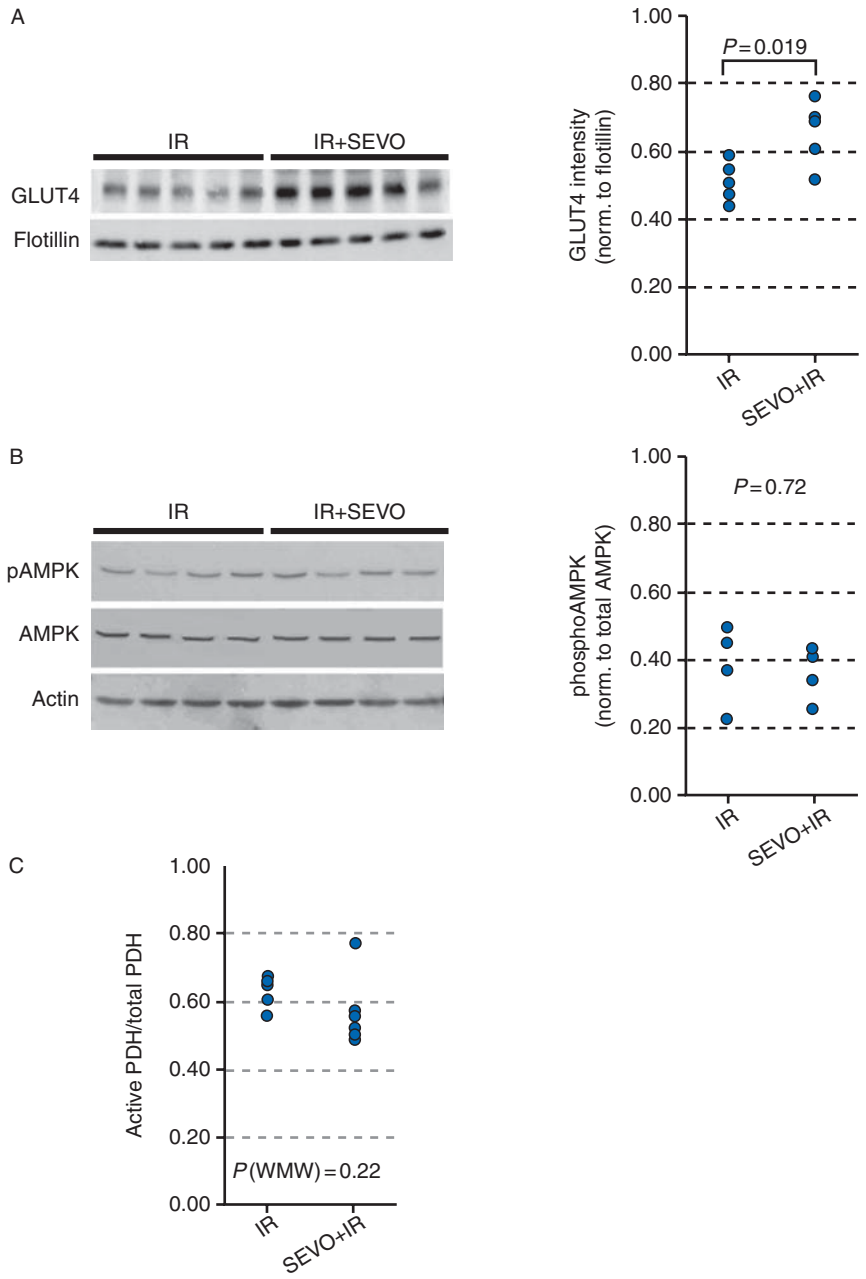

Fig 5 GLUT4 expression, AMPK, and PDH activities in untreated hearts (IR) or in hearts exposed to sevoflurane (2 vol%, IR+SEVO). (A) Representative western blots ($n=5$ per group) of GLUT4 expression in lipid rafts and quantification to flotillin. (B) Representative western blots of AMPK and activity estimated from the ratio of pAMPK and total AMPK. α-Actin was used as a loading control ($n=4$ for each group). (C) PDH activity calculated as active over total PDH ($n=5$ for each group). *P*-values were computed using Student's *t*-test or the Wilcoxon–Mann–Whitney test (WMW).

Reduced mitochondrial respiration as evidenced by lower acetyl-CoA turnover in sevoflurane-treated hearts resembles a state of 'metabolic hibernation', previously identified as a typical feature of the preconditioned protected state of the heart.[38] Volatile anaesthetics such as sevoflurane activate ATP-dependent potassium channels, a key player in the preconditioning process,[8] which is tightly regulated by intermediary metabolism. In this regard, it is interesting to note that sevoflurane did not alter cardiac glycogen stores, despite the well-known activation of glycogen synthase kinase-3β by volatile anaesthetics.[39] However, the glycogen-salvaging effects by sevoflurane may be only seen after more extended ischaemic periods. Although previous work suggests that brief sevoflurane preconditioning increases phosphorylation of AMPK during reperfusion,[40] in our protocols where sevoflurane was administered during the entire

experimental protocol, no increase in AMPK phosphorylation was observed. Nonetheless, our data lend further support to the concept of a tight interplay among energy metabolism, preconditioning, and cardioprotection.

Our study has several limitations. In our analyses, we focused on the intrinsic metabolic effects of the two anaesthetics and Intralipid. Hence, we compared sevoflurane-treated hearts with untreated control hearts, and since Intralipid-treated hearts also exhibited some changes in metabolites, propofol-treated hearts were compared with both untreated and Intralipid-treated hearts as described previously.[3] A direct comparison between sevoflurane- and propofol-treated hearts would not have allowed delineating the specific metabolic effects of these pharmacological agents and linking them to cardiac function and Ca^{2+} overload. Although we did not observe significant cardioprotection in propofol-treated hearts in our experiments, we cannot exclude some protection with 100 μM propofol or other concentrations in other experimental settings. In fact, propofol is known to have Ca^{2+} channel-blocking activities and baseline and changes in diastolic Ca^{2+} concentrations tended to be lower in the propofol group (Fig. 2; Supplementary Table S2). However, ischaemic Ca^{2+} concentrations and changes in systolic Ca^{2+} concentrations were not different from untreated hearts. Irrespectively, the failure of propofol to improve post-ischaemic cardiac function is unlikely to be due to its negative inotropic effects since propofol (100 μM) did not suppress cardiac contractility under aerobic conditions. Rather the increased Ca^{2+} overload in propofol-treated hearts suggests the ischaemic damage as cause for the poor recovery. Finally, since the effects of propofol on rat myocardium may be different from those on human myocardium, we should be careful in extending the current results to human hearts.

In conclusion, this study demonstrates that enhanced glucose uptake via GLUT4 fuels fast recovery from Ca^{2+} overload after ischaemia–reperfusion injury in sevoflurane-treated hearts. Our current experimental results confirm previous clinical findings[4 5 41] in a well-controlled physiological model and shed new light on the potentially cardioprotective metabolic effects of sevoflurane.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

Conflict of interest

None declared.

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